0022-202X/78/7005-0246$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 70:246–249, 1978

Vol. 70, No. 5
Printed in U.S.A.

CYCLIC NUCLEOTIDE-PHOSPHODIESTERASE IN THE UNINVOLVED AND INVOLVED SKIN OF PSORIASIS

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In the present study we have compared cyclic nucleotide-phosphodiesterase activities and affinity of phosphodiesterase for substrates (Km) in enzyme preparations obtained from the involved and uninvolved skin of psoriatic patients. With crude skin homogenates we consistently obtained two Km values (high and low) for both the involved and uninvolved, and both Km values were nearly identical between the involved and uninvolved. The same conclusion is also drawn from the Km determinations with partially purified preparations. Cyclic AMP-phosphodiesterase activities with crude homogenates showed no statistically significant differences between the involved and the uninvolved skin. However, when cyclic AMP- and cyclic GMP-phosphodiesterase activities were compared with a highly sensitive assay method in "pure" epidermal samples, which were microdissected free from stratum corneum, dermis and skin appendages, the involved skin contained 40% more activity of the low Km enzyme and 100% more of the high Km enzyme of both cyclic AMP- and cyclic GMP-phosphodiesterase. It is suggested that this may be due to a higher proportion of germinative cells in the lesional epidermis.

One of the biochemical characteristics found in psoriatic lesion appears to be the sluggish response of its cyclic AMP system to external stimuli. For example, the cyclic AMP system in psoriatic skin cannot respond well to either β-adrenergic agonist [1–3] or prostaglandin [4,5], i.e., in an in vitro system the involved skin accumulates much less cyclic AMP intracellularly as compared with the uninvolved skin on the addition of either drug. Since the cyclic AMP level in a cell depends on a balance of a synthetic (adenylate cyclase) and degradative (cyclic AMP-phosphodiesterase [PDE]) enzyme, the failure to accumulate cyclic AMP may be sought for as an abnormality in either one or both enzyme systems. Available data [1–5] appear to support the concept that the major abnormality in the cyclic AMP system lies in the defective adenylate cyclase system, however, the paucity of information on the degradative enzyme in psoriasis, has encouraged us to study PDE comparatively in the involved and uninvolved skin of psoriasis.

MATERIALS AND METHODS

Human skin samples were obtained from adult male patients with well-developed psoriatic lesions. No active treatment was given for at least 7 days. The skin samples were taken with Castroviejo keratome (Storz Instrument, St. Louis, Mo.) adjusted to a 0.3-mm setting without the use of anesthesia. The histological examination generally revealed that the samples were predominantly epidermis with little dermis, but the degree of the dermal contamination was variable. About 20 to 50 mg skin was homogenized in double distilled water at the final concentration of 2% (w/v) just before the enzyme assays. The PDE activities were measured by the method described by Scott and Solomon [6] with minor modifications [7]. Protein concentration was measured by the method of Lowry et al [8].

The PDE activities were also assayed with a micro technique as follows: skin samples obtained by 4-mm punch were immediately frozen, cut at 20 μ thick at −30°C and freeze dried [9]. A few micrograms of "pure" epidermis was microdissected under a stereomicroscope, weighed on a "quartz microbalance" [9], and placed at the bottom of a home-made test tube, 2 mm in inner diameter and 25 mm in length. Ten μl each of the reagent mixture which was the same as the one used for the semi-micro assay system [7], was added and the tubes were incubated at 37°C for 1 hr. The reaction was stopped by the addition of 2 μl of 2 N HCl, and 1 μl of the reaction mixture was spotted on thin-layer plate for analysis of the products as described previously [7].

Cyclic (^{3}H) AMP (specific activity: 27.7 Ci/mmole) and cyclic (^{3}H) GMP (specific activity: 11 Ci/mmol) were purchased from New England Nuclear (Boston, Mass.). Their radiochemical purity was checked regularly on thin-layer chromatography [6,7]. Thin-layer cellulose plates were products of Analtech (Newark, Del.). All other chemicals and reagents were obtained from Sigma (St. Louis, Mo.), except for Omnifluor and BBS III which were products of New England Nuclear and Beckman (Anaheim, Calif.) respectively.

RESULTS

I. Comparison of PDE Activities in Homogenates Made from the Uninvolved and Involved Tissue

The PDE activities were compared in 7 cases at a high and low Km substrate level (Table I). In 3 cases the activities in the uninvolved were higher than those in the involved, and in the other 3 cases this tendency was reversed. The quantitated average of 7 cases showed an increase in the involved skin (55% for high Km and 11% for low Km) on a wet weight basis. These wet weight differences were not significant, and there was essentially no difference on a protein basis.

The reason for the large variability of the data can be in part, attributed to the histological complexity of psoriatic lesions. Depending on the sampling with a keratome, the lesion may include variable amounts of keratin, dermis, inflammatory cells and exudates, capillaries, skin appendages, etc. In fact, the standard error or the mean of the uninvolved ranges from 5 to 15%, but those of the involved from 17 to 40% (Table I).

II. Comparison of apparent Km of PDE in the Uninvolved and Involved Skin of Psoriasis

The apparent Km values were computed from Lineweaver-Burk plots. Since the analytical method for Km is independent of the enzyme (homogenate) concentration used, the variability of the experimental data due to the contamination of keratin, etc., is much less.

A typical graphical analysis of Km is shown in the Figure. Both the uninvolved and involved skin showed 2 identical Kms: a high Km of 6×10^{-5} M and a low of 6×10^{-6} M. These 2 Km values suggest either the presence of 2 separate enzymes acting on the same substrate or of a single enzyme with unusual

Manuscript received August 18, 1977; accepted for publication October 30, 1977.

This study was supported in part by a grant AM17179 of the National Institutes of Health and the Dermatology Foundation of Miami, and in part by the Arthur O. Wellman Fund.

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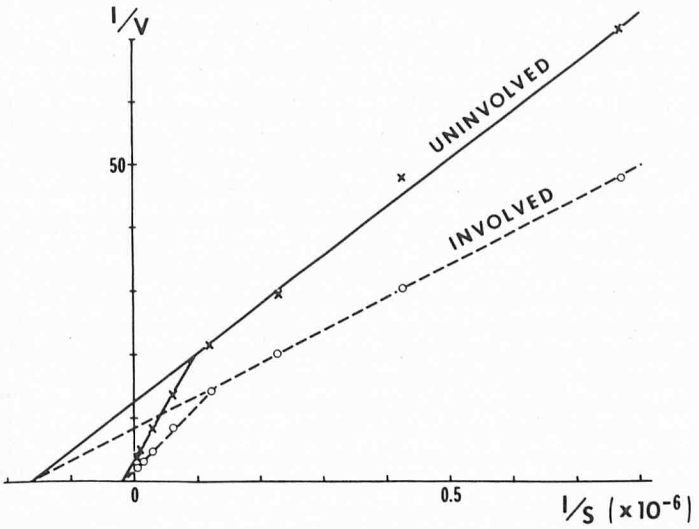
Abbreviation:
PDE: cyclic AMP-phosphodiesterase

TABLE I. *Cyclic AMP-phosphodiesterase activities and apparent Km values in the uninvolved and involved skin of psoriasis*

	High Km		Low Km	
	Uninvolved	Involved	Uninvolved	Involved
	(pmoles/min/mg wet weight)			
PDE activity	35.9 ± 4.88	55.8 ± 18.0	1.60 ± 0.11	1.78 ± 0.31
	(pmoles/min/mg protein)			
PDE activity	290 ± 35	250 ± 94	13.8 ± 0.53	10.2 ± 1.7
Km	5.55 ± 0.30 ($\times 10^{-5}$)	5.45 ± 0.62	5.33 ± 0.27($\times 10^{-6}$)	5.50 ± 0.35

The enzyme activities represent average values of 7 cases (duplicate determination each) with standard errors of the mean. The substrate cyclic AMP levels were 75 μM for high Km and 0.75 μM for low Km enzyme assays. The Km values are averages of 4 cases ± SE. In 1 case partially purified preparation was used (for detail see text).



Lineweaver-Burk plots for the determination of apparent Km values for the uninvolved and involved skin of psoriasis. For experimental detail see text.

TABLE II. *Cyclic AMP-phosphodiesterase activities in the microdissected "pure" epidermis from the involved and uninvolved skin of psoriasis*

	Uninvolved	Involved	*P*
Low Km enzyme	pmoles/min/mg dry weight		
Case A	8.8 ± .68	10.7 ± 1.3	NS
Case B	12.5 ± .48	19.2 ± .49	$p < 0.01$
Case C	12.3 ± .53	16.8 ± .86	$p < 0.01$
Case D	12.4 ± .52	16.6 ± .38	$p < 0.01$
Case E	9.6 ± .69	13.8 ± .94	$p < 0.01$
Case F	9.9 ± .87	14.6 ± 1.1	$p < 0.01$
Case G	9.4 ± .56	12.1 ± .64	$p < 0.01$
Average	10.7 ± .62	14.8 ± .82	$p < 0.01$
High Km enzyme			
Case A	173 ± 9.2	182 ± 14	NS
Case B	255 ± 13	604 ± 76	$p < 0.01$
Case C	195 ± 14	315 ± 19	$p < 0.01$
Case G	191 ± 21	367 ± 12	$p < 0.01$
Average	203 ± 14	367 ± 30	$p < 0.01$

The substrate levels for the low Km enzyme assays were 0.87 μM for cases A–D and 0.9 μM for cases E–G, and those for the high Km enzyme assays 52.7 μM for cases A and C and 96.0 μM for cases B and G. "*p*" values were for the differences between the enzyme activities in the uninvolved and involved (NS: Not significant). Values are expressed with ± SE (n = 6).

kinetics. With respect to human skin the former appears to be the case, since the occurrence of multiple forms of PDE in pig skin has been demonstrated [7]. The Km values were further computed from skin homogenates obtained from 2 other psoriatic cases (Table I). Both high and low Km values for the uninvolved and involved were almost identical: the data indicate the affinity of PDE to substrate is unchanged in the psoriatic lesion.

In one experiment, we purified the enzyme partially by ammonium sulfate precipitation [10]. The 2,000 ×g supernatant fractions from the involved and uninvolved were subjected to 20% saturation with ammonium sulfate and the resultant precipitates were discarded. Ammonium sulfate was added further and the fractions precipitated at 40% saturation were used for Km determinations immediately after dialysis. Again no significant difference in both high and low Km values between the involved and uninvolved was noted.

III. *Comparison of PDE Activities in Microdissected "Pure" Epidermis from the Involved and Uninvolved Skin of Psoriatic Patients*

In Tables II and III we summarize the results of PDE assays in "pure" epidermis, which were dissected out under a stereomicroscope free from keratin, dermis and other skin appendages. Each figure represents an average of 6 determinations. The overall average standard errors of the mean were 6.43% for cyclic AMP-PDE and 8.54% for cyclic GMP-PDE.

The average activity of the low Km cyclic AMP PDE in the involved was 14.8 and that in the uninvolved 10.7 pmoles/min/mg dry weight, respectively. The involved had 40% more enzyme activity and this difference was significant ($p < 0.01$) by either unpaired or paired *t*-test. The differences in the enzyme activities in individual cases were all highly significant except case A (Table II, *p* values). The difference between the involved and uninvolved was larger for the high Km enzyme than the low Km enzyme, i.e., the average difference was 80% and the difference was significant.

With respect to cyclic GMP-PDE almost identical results were obtained (Table III). Averages of low Km enzyme activities were 52.8 and 38.5 pmoles/min/mg dry weight for the involved and uninvolved respectively. The involved had about 40% more activity than the uninvolved. This difference was also statistically significant. The differences in the high Km enzyme activities between the involved and uninvolved were clear, i.e., the activities were nearly doubled in the involved with the exception of case A, in which we noted increased variability in the enzyme activities for unknown reasons.

Table IV summarizes the results of PDE assays in further microdissected "upper" and "lower" epidermis. The results clearly indicate that the lower epidermis has much higher activity than the upper epidermis does. The "lower epidermis" of the involved skin probably contained almost entirely germinative cells, while the "lower epidermis" of the uninvolved probably still contained more "prickle" than "basal" or germinative cells. The enzyme activities of the uninvolved (lower epidermis) approached those of the germinating cells in the involved. Thus, the overall increase in PDE activities in psoriatic lesional epidermis appears to reflect the increase in the dividing cell populations in the involved.

DISCUSSION

PDE activities in the involved and uninvolved skin of psoriasis have been compared by 2 groups previously. Härkönen, Hopsu-Havu, and Raji [11] reported about 3/4 less cyclic AMP-PDE activity in the involved skin as compared with that in the uninvolved at the substrate level of 5 mM (i.e., a high Km enzyme). Voorhees et al [12], however, reported no essential difference when the PDE activities were compared with crude homogenate from the involved and uninvolved. Our PDE assays with crude homogenate (c.f. Table I) showed no significant difference between the involved and uninvolved. The results by all 3 groups had large variations as reflected by large standard errors of means being 20 to 30% (c.f. references 11,12, and Table I of this paper). This appears to be due to the histological complexity of psoriatic lesion and to the difficulty of obtaining homogeneous epidermal samples by a keratome. The "psoriatic" lesional keratome slice contains large amounts of stratum corneum, dermal rete ridges, blood vessel net works, and dermal infiltrates. Even the "normal" keratome slice may contain 10 to 50% dermal contamination, which makes the PDE activity lower. Thus, we have developed a highly sensitive micromethod for PDE assay so that "pure" microdissected epidermis could be assayed and compared.

Our microassay done on pure epidermis from lesions and from uninvolved skin of the same patients demonstrated a clear increase in every case in the lesion. This was true of both cyclic AMP PDE and cyclic GMP PDE. In most cases the lesional epidermis had almost twice as much PDE as the uninvolved epidermis. When the epidermis was divided, however, into an upper and a lower portion, it became evident that both cyclic AMP PDE and cyclic GMP PDE are about twice as active in the lower slices. The data presumably reflect increased activity in the proliferating cell as compared with the nonproliferating. In addition a significantly higher portion of the cyclic GMP PDE is retained in the upper layers of the lesion than in the uninvolved. This could be due to the rapid transition in psoriatic lesions from germinative to upper layers and a lack of time for normal loss of PDE activity with maturation of the cells. On this basis, the increased PDE activity found in the lesion may only represent an increased number of germinative cells in the lesion and retention of high PDE activity during the more rapid progression from a germinative to a superficial location.

As far as the kinetic parameters of PDE are concerned, Voorhees et al [12] reported no difference in Km values between the involved and uninvolved with whole homogenates as enzyme source: The data coincide with ours in this study. Voorhees et al [13,14], however, later reported increased low Km value in the involved with 17,000 g supernatant fraction (but no difference in Km with the 17,000 ×g pellet fraction as enzyme source). The latter data apparently contradict ours, i.e., we were unable to find any difference in Km even after partial purification of PDE from the involved and uninvolved skin of psoriasis. All of their data are cited in review articles [12–14] without recording of details of experimental procedures and results. Hence, further comparison of data may not be meaningful at present. Voorhees et al also stated that V max values for the involved are higher than those for the uninvolved [13]. Although our kinetic data appear to coincide with theirs, we have not done further studies of the V_{max} values, since V_{max} values with crude enzyme systems have limited value, particularly in the case of psoriasis where the crude enzyme consists of a variety of inhomogeneous proteins.

The possibility of a role played by an endogenous Ca^{++} activator [15,16] has been raised in regard to PDE activity. Preliminary experiments demonstrated that adding ethylene glycol tetraacetic acid (EGTA) in the assay mixture to remove Ca^{++} caused only a 10 to 20% decrease in both the involved and uninvolved. This is against a significant role for the activator or Ca^{++} dependent PDE in psoriatic lesions, but further work using purified PDE must be done before this can be regarded

TABLE III. *Cyclic GMP-phosphodiesterase activities in the microdissected "pure" epidermis from the involved and uninvolved skin of psoriasis*

	Uninvolved	Involved	p
Low Km enzyme	pmoles/min/mg dry weight		
Case A	33.7 ± 2.6	49.0 ± 5.1	$p < 0.05$
Case B	35.6 ± 2.3	55.2 ± 4.9	$p < 0.01$
Case C	41.6 ± 4.9	58.1 ± 2.1	$p < 0.01$
Case D	55.3 ± 2.1	64.1 ± 6.2	$p < 0.01$
Case F	31.6 ± 4.3	45.6 ± 2.9	$p < 0.02$
Case G	33.3 ± 3.8	44.7 ± 2.8	$p < 0.05$
Average	38.5 ± 3.3	52.8 ± 3.1	$p < 0.01$
High Km enzyme			
Case A	187 ± 31	214 ± 21	NS
Case B	279 ± 20	516 ± 41	$p < 0.01$
Case C	240 ± 22	560 ± 38	$p < 0.01$
Case G	209 ± 16	339 ± 34	$p < 0.01$
Average	228 ± 22	407 ± 34	$p < 0.01$

The substrate level for the low Km enzyme was 3.35 and the levels for the high Km enzyme were 76, 76, 109 and 102 μM for the cases A, B, C and G respectively. "p" values were for the differences between the enzyme activities in the involved and uninvolved (NS: Not significant). Values are expressed with ± SE (n = 6).

TABLE IV. *Phosphodiesterase activities in the upper and lower epidermal layers microdissected from the involved and uninvolved skin of psoriasis*

	Uninvolved		Involved	
	Upper	Lower	Upper	Lower
Cyclic AMP-PDE				
Case A	8.2 ± .96	15.4 ± 1.4		
Case C			7.5 ± .98	24.6 ± 2.2
Case D			8.6 ± .80	18.9 ± 2.1
Case E			11.0 ± .53	15.5 ± .53
Case F			12.9 ± 1.1	19.1 ± 2.4
Case G	7.1 ± .43	11.7 ± .76	11.8 ± 1.4	20.8 ± 1.8
Cyclic GMP-PDE				
Case A			26.6 ± 3.5	48.6 ± 5.4
Case C	8.6 ± .72	41.3 ± 10		
Case E	9.1 ± 2.7	31.4 ± 3.8	27.7 ± 2.9	55.6 ± 6.8
Case F			35.9 ± 3.4	69.0 ± 4.3

The substrate level for cyclic AMP-PDE was 0.9 μM and that for cyclic GMP-PDE 3.35 μM. Each figure is an average of 5 to 6 determinations (pmoles/min/mg dry weight ± SE). Samples from the involved lower epidermis in cases A, C and D were dissected from epidermal ridges extending downward into dermis. The differences between the upper and lower values are all highly significant ($p < 0.01$) except case G, the uninvolved ($0.02 < p < 0.05$).

as a firm conclusion. The overall results suggest (1) PDE in the involved epidermis appears to have nearly the same characteristics of that in the uninvolved and (2) the apparent increase in the PDE activities in the involved epidermis may be merely a consequence of the increased population of proliferating cells.

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